MAPPING FUNGAL RESISTANCE GENES IN GRAPE

Gomba rezisztencia gének térképezése szőlőben

K62535

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INTRODUCTION AND OBJECTIVES OF THE PROJECT

Production of grape varieties of high quality, at the same time resistant to fungal diseases is one of the most crucial goals of grape breeding. The aim of the project entitled "Mapping fungal resistance genes in grapevine" (K62535) was to identify and validate marker alleles linked to resistance genes against downy (DM) and powdery (PM) mildew (*Plasmopara viticola* Berk. et Curtis ex. de Bary Berl. et de Toni) and *Erysiphe necator* Schwein. / *Uncinula necator*). Molecular markers are efficient tools in mapping, finding major and minor QTL-s contributing to resistance and cloning of these genes.

The main goals of the project were:

- Application of RGA (Resistance Gene Analogue) CAPS (Cleaved Amplified Polymorphic Sequences) and SSR markers linked to PM and DM resistance genes for analyzing hybrid mapping populations, deriving from different interspecific crosses of *Vitis vinifera* with *Muscadinia rotundifolia. Muscadinia rotundifolia* carries dominant *RUN*1 *and RPV*1 genes providing resistance against PM and DM infections, respectively.
- Analysis of Central-Asian *Vitis vinifera* varieties such as Dzhandzhal kara and Kishmish vatkana highly resistant to powdery mildew. These two Central–Asian varieties belong to the small group of grape cultivars exhibiting resistance against powdery mildew, while the traditional, high quality European *Vitis vinifera* cultivars are susceptible to the mildew infections.
- It was our important aim to investigate the molecular basis of powdery mildew resistance of Kishmis vatkana examining its hybrid combinations to determine, whether the PM resistance gene of Kishmish vatkana is identical or not with *RUN*1 PM resistance gene of *Muscadinia rotundifolia*. Furthermore in the case of diversity, to localize the new resistance gene in the linkage groups of grape.
- Characterisation of biodiversity of old and registered grapevine varieties with SSR markers (SSR: Simple Sequence Repeat).
- Comparison of several old and new grapevine varieties with SSR markers linked to fungal resistance genes identified in *Muscadinia rotundifolia* (Powdery mildew: *RUN*1-*Uncinula necator*, downy mildew: *RPV*1*: Plasmopara viticola)* and *Vitis vinifera* Kishmish vatkana.

EXPERIMENTAL APPROACHES AND RESULTS

MARKER ANALYSES OF PROGENY CARRYING THE *RUN*1/*RPV*1 RESISTANCE GENE FROM *MUSCADINIA ROTUNDIFOLIA*

Fungal disease resistance has been a cardinal point of grape breeding since the 19th century when pathogens such as powdery mildew (*Uncinula or Erysiphe necator* Schwein) and downy mildew (*Plasmopara viticola* Berk. et Curtis ex. de Bary Berl. et de Toni) were carried to Europe from North America. European viticulture faced a new challenge and a significant environmental risk factor because traditional cultivars of *Vitis vinifera* origin do not carry any resistance to the mildew fungi, therefore repeated fungicide applications have been necessary during the vegetation period. All sources of resistance providing high or partial resistance to these pathogens are low-quality wild species, therefore introgression of the resistance genes from the wild species such as *V. rupestris, V. berlandieri, V. labrusca, V. rubra* requires many back-crosses with *Vitis vinifera* in order to produce high quality *vinifera* cultivars (EIBACH *et al.* 1989; DOLIGEZ *et al*. 2002; FISCHER *et al*. 2004). Combining resistance, e.g., from American wild *Vitis* species, with good wine qualities of *Vitis vinifera* L. became an important strategy of grapevine breeding. *Muscadinia rotundifolia* was described as totally resistant to powdery mildew species (BOUBALS 1959; OLMO 1971; STAUDT and KASSEMEYER 1995; PAUQUET *et al*. 2001). The discovery of *RUN*1 gene in the American muscadine grape (*M. rotundifolia* Michx. Small) initiating effector-triggered immune responses against PM opened new possibilities in grapevine breeding (Bouquet, 1986, Dry et al., 2010).The *RUN*1 locus was mapped into the linkage group 12 and it encompasses a string of resistance genes, three of which were found closely linked to a marker co-segregating with resistance (PAUQUET et al., 2001, DONALD et al., 2002). In spite of the fact, that the chromosome numbers are different in *Vitis* (2n=38) and *Muscadinia* (2n=40), the hybridization of these two species succeeded eventually (PATEL *et al*. 1955; OLMO 1971; BOUQUET 1980; 1986). The total resistance to powdery mildew originating from *Muscadinia rotundifolia* is controlled by a single dominant locus (BOUQUET 1986), called *RUN*1 (for **R**esistance to ***U**ncinula **N**ecator* 1). It was introduced into *Vitis vinifera* genome using a pseudo-backcross strategy aiming at the production of good quality grape varieties resistant to powdery mildew (BOUQUET 1986).
Molecular markers - significant in mapping resistance genes - closely linked to this locus have been identified (PAUQUET *et al*. 2001; DONALD *et al.* 2002), allowing MAS (marker assisted selection) to be used in a breeding program and in the positional cloning of the *Run*1 powdery mildew resistance gene (BARKER *et al.* 2005 a).
Our research aimed at the application and validation of molecular markers linked to *RUN*1 powdery mildew resistance locus in BC_5 individuals originating from the (*Muscadinia rotundifolia* L. *x Vitis vinifera* L.) (VRH 3082-1-42) BC_4 x Cardinal cross and BC_4 x Kishmish moldavskij (KOZMA 2002) based on the results published by DONALD *et al*. (2002) and BARKER *et al*. (2005 b).
The scheme of the production of BC_5 progeny is and the position of *RUN*1 linked markers is illustrated in Figure 1 and 2, respectively.

*RUN*1 ANALYSES IN BC_4 X CARDINAL HYBRID FAMILY

As a first step 20-20 BC_5 plants of the 02-2 hybrid family (*Muscadinia rotundifolia x Vitis vinifera*) BC_4 x *Vitis vinifera* cv. Cardinal were selected according to powdery mildew symptoms on leaves and were tested with a CAPS / RFLP-PCR marker (GLP1-12P1-P3 primers; DONALD *et al.* 2002). One 870 bp DNA fragment was amplified both in healthy and susceptible plants. As it was expected according to DONALD *et al.* (2002) discrimination between symptomless and infected individuals was only possible by the digestion of the PCR

product. *EcoR*I cleaved the DNA amplicon of the symptomless leaves into two pieces (670 bp and 200 bp), while it did not split the PCR product of the susceptible samples.

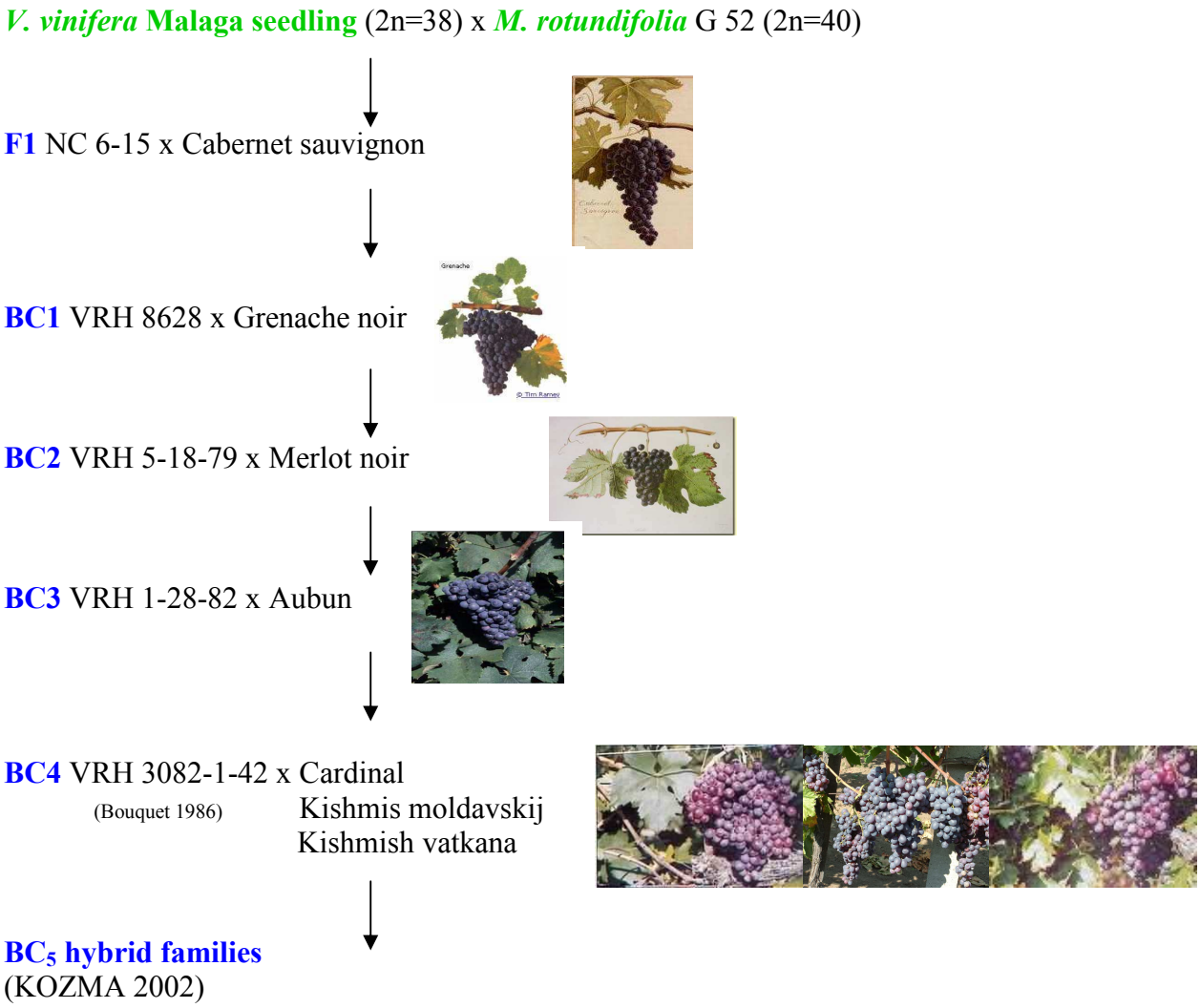

Figure 1. Production scheme of BC_5 hybrid families with a pseudo-backcross method.

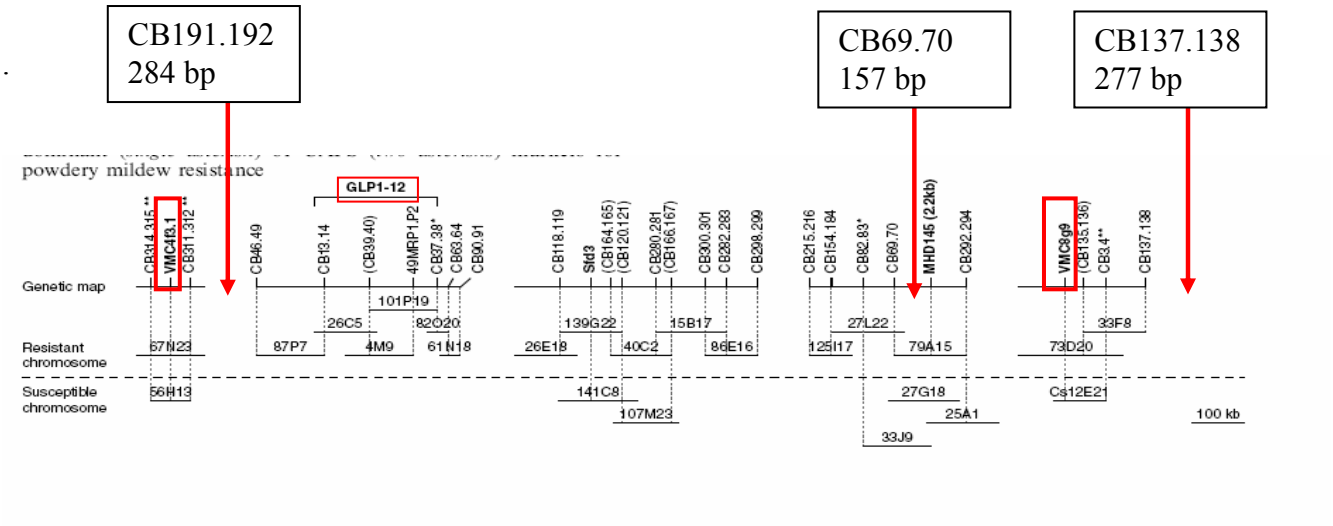

Figure 2. Position of markers used in the *RUN*1 analyses (after BARKER et al. 2006 and DRY personal communication).

Based on the results obtained with the 20-20 plants, altogether 142 seedlings from the BC_5 02-2 hybrid family were screened with the GLP1-12P1-P3 and two microsatellite primers. Involvement of microsatellite primers (VMc8g9 and VMC4f3.1 linked to PM linked to *RUN*1 locus) aimed at simplifying the screening process. Additionally analysis with microsatellite primers provided a way to monitor outcrosses, too. As a result, lines carrying „alien" alleles were excluded from further analyses. Due to these non-parental allele combinations and incidental uncertain phenotyping data of 129 lines were included in the evaluation. Table 1 summarizes the data concerning the two parents (BC4 VRH 3082-1-42 and Cardinal) and the 129 progeny.

The 67:62 ratios of symptomless and susceptible lines correspond to the 1:1 Mendelian segregation. Similarly, the genotypic segregation determined by the GLP1-12P1-P3 and the microsatellite markers follows Mendel's law (Table 1). However, in the case of all three molecular markers recombinants were obtained: powdery mildew symptomless individuals, whose GLP1-12P1-P3 PCR amplicons remained uncut after *Eco*RI digestion or the microsatellite alleles coupled with the resistance were missing from them. In the case of VMC4f3.1 microsatellite a 186 bp allele (Table 1), while in case of VMC8g9 a 160 bp allele proved to be a powdery mildew resistance linked marker (Table 1). These two microsatellite loci are mapped at the opposite ends of *RUN*1 locus, in an RGA region (BARKER *et al*. 2005). VMC4f3.1 showed the highest, and PCR-RFLP the lowest recombination frequency. A few symptomless lines having sensitive allele sizes of 167 bp (VMC8g9) or 184 bp (VMC4f3.1) were also identified.

T a b l e 1

Comparison of the phenotyping results for powdery mildew symptoms and genotyping with molecular markers (shaded numbers indicate the „resistant allele" sizes)

Variety/ population	Phenotype		Molecular markers					
	Symptom-less	Suscep-tible	GLP1-12P1-P3 Digestibility of PCR fragment with *Eco*RI enzyme		VMC4f3.1 alleles (bp)		VMC8g9 alleles (bp)	
	R	S	R yes	S no	R 186	S 184	R 160	S 167
Cardinal	-	+	-	+		164:164		179:179
VRH 3082-1-42 BC_4	+	-	+	-	184:186		160:167	
BC_5 02-2 hybrid family	67	62	66	63	164:186	164:184	160:179	167:179
					61	68	66	63
Ratio of recombinants			1/129=0.007		13/129=0.100		5/129=0.038	

Results and Conclusions

In spite of the fact that the linkage of the applied molecular markers proved to be lower than 100%, these markers can be successfully applied in MAS, since 90-99% of the plants selected in this way will carry the dominant *RUN1* powdery mildew resistance gene. With regard to rapidity and efficiency the VMC8g9 proved to be the most favourable of the three markers

(GLP1-12P1-P3, VMC4f3.1 and VMC8g9) because the discriminative 160-167 bp fragments can be separated on an agarose gel following a simple PCR allowing of the routine analyses of many samples at the same time. Because of the only 2 bp difference between the resistant and sensitive alleles in the case of VMC4f3.1, this marker is not suitable for reliable routine analysis.

Publications relevant to the topic

Molnár S., Galbács Zs., Halász G., Hoffmann S., Kiss E., Kozma P., Veres A., Galli Zs., Szőke A., Heszky L. 2007. Marker assisted selection (MAS) for powdery mildew resistance in a grapevine hybrid family. Vitis 46: 12-213.

Molnár S., Galbács Zs., Halász G., Hoffmann S., Veres A., Szőke A., Galli Zs., Szádeczky-Kardoss B. Kozma P., Kiss E., Heszky L. (2007): Liszthármat ellenálló és fogékony genotípusok szelekciója molekuláris markerekkel. Debreceni Egyetem Agrártudományi Közlemények, *Acta Agraria Debreceniensis* 2007/27:100-104.

*RUN*1/*RPV*1 ANALYSES IN BC_4 X KISHMISH MOLDAVSKIJ HYBRID FAMILY

50 seedlings of BC4 x Kishmish moldavskij unselected for PM infections (summer DM leaf symptoms due to natural infection were given by Pál Kozma and Sarolta Hoffmann) were analyzed first with the VMC8g9, and VVMC1g3.2 primers.

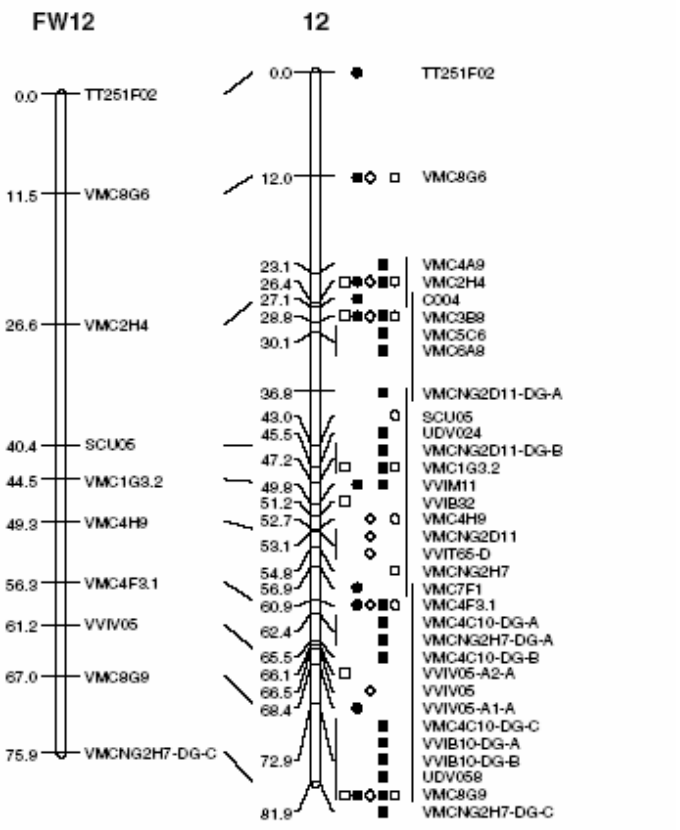

Figure 3: Position of VMC1g3.2 and VVIm11 marker loci in LG 12 (DOLIGEZ et al. 2006).

At the beginning we started to use VMC4f3.1, but because of the 2-bp difference between the *RUN*1-linked allele and its homologue (186 vs. 184 bp, respectively) we discontinued the use of this marker. VMC8g9 marker was found to consistently co-segregate with PM resistance phenotype in VRH3082-1-42 x *V. vinifera* cv. Cabernet Sauvignon while VMC1g3.2 is in a 4.4 cM distance from it (BARKER et al. 2005). VVMC1g3.2 was described as *RPV*1 marker by WIEDEMANN-MERDINOGLU (2006). In the cross of BC_4 x Kishmish moldavskij, Kishmish moldavskij is sensitive to PM and DM, therefore resistant progeny carries the *RUN*1/*RPV*1 dominant genes and marker alleles of BC_4, consequently resistant genotypes are as follows: 160-174 bp, 160-160; 294-294; 122-128, 122-142 bp (Table 2). As it can be seen in Table 2. the 160 bp marker allele of VMC8g9 appeared in Kishmish moldavskij, therefore the presence of *RUN*1 gene is convincing only in 160-160 bp homozygous genotypes. Application of other markers is necessary to analyze the 160-174 bp heterozygous plants. Beside VMC1g3.2 we chose another marker VVIm11 relatively close to the VMC1g3.2 locus (Figure 3). VVim11 has not been published yet to be used as a *RPV*1 marker; we applied it first to distinguish the sensitive and resistant hybrids of BC_4 x Kishmish moldavskij. We wanted to check cosegregation of VMC8g9 and VVMC1g3.2 with VVim11.

T a b l e 2

Marker results in the BC_4 x Kishmish moldavskij family

Variety	*RUN1/RPV1*					
	VMC8g9	*Number of individuals*	VVIm11	*Number of individuals&*	VMC1g3.2	*Number of individuals*
BC_4	160-167		272-294		122-142	
Kishmish moldavskij	160-174		294-294		128-142	
*Susceptible progeny	160-167 167-174	17	272-294	9	142-142 128-142	18
*Symptomless progeny	160-174 160-160	31	294-294	12	122-142 122-128	29
Recombinants?		2		2(&)		3

*Susceptible and Symptomless categories were set up according to the DM symptoms appeared in field conditions. Shaded numbers indicate the resistance-specific alleles.
&: 23 samples were tested only with VVIm11. (&): These 2 plants in the "Recombinant?" category gave congruent results with VMC8g9, too.

Results and Conclusions

In the case of VMC8g9 marker the presence of *RUN*1 gene is convincing only in 160-160 bp homozygous genotypes. Application of other markers is necessary to analyze the 160-174 bp heterozygous plants.

Phenotyping under PM and DM provocative conditions and increase the number of plants analyzed should be necessary to decide whether the genotypes out of susceptible or

symptomless categories are true recombinants and to determine the cosegregating type of the newly applied VVIm11 marker with VMC8g9 and VMC1g3.2.

Publications relevant to the topic

Katula-Debreceni D., Veres A., Szőke A., Lencsés A.K., Kozma P., Hoffmann S., Kiss E. 2010. Marker-based selection for powdery mildew resistance genes in different grape hybrid families. *Submitted*

*RUN*1/*RPV*1 ANALYSES OF BC_3 SELF-FERTILIZED PROGENY

From the combinations illustrated by Figure 4. Research Institute of Viticulture and Enology used VRH1-1182 and VRH5 8-82 as BC3s in the cross programs. Any of them should contain the *RUN*1/*RPV*1 (R) gene in heterozygous status, so their self-fertilization should result in RR:Rr genotypes in 1:2 ratio. Analyzing the healthy progeny (56 plants) with VMC8g9, VMC1g3.2 and VVIm11 markers we obtained the expected genotypes in the case of VMC8g9 and VVIm11 loci, but not at all in VMC1g3.2. However the segregation ratio did not correspond to the expected 2:1 in any of these latter loci.

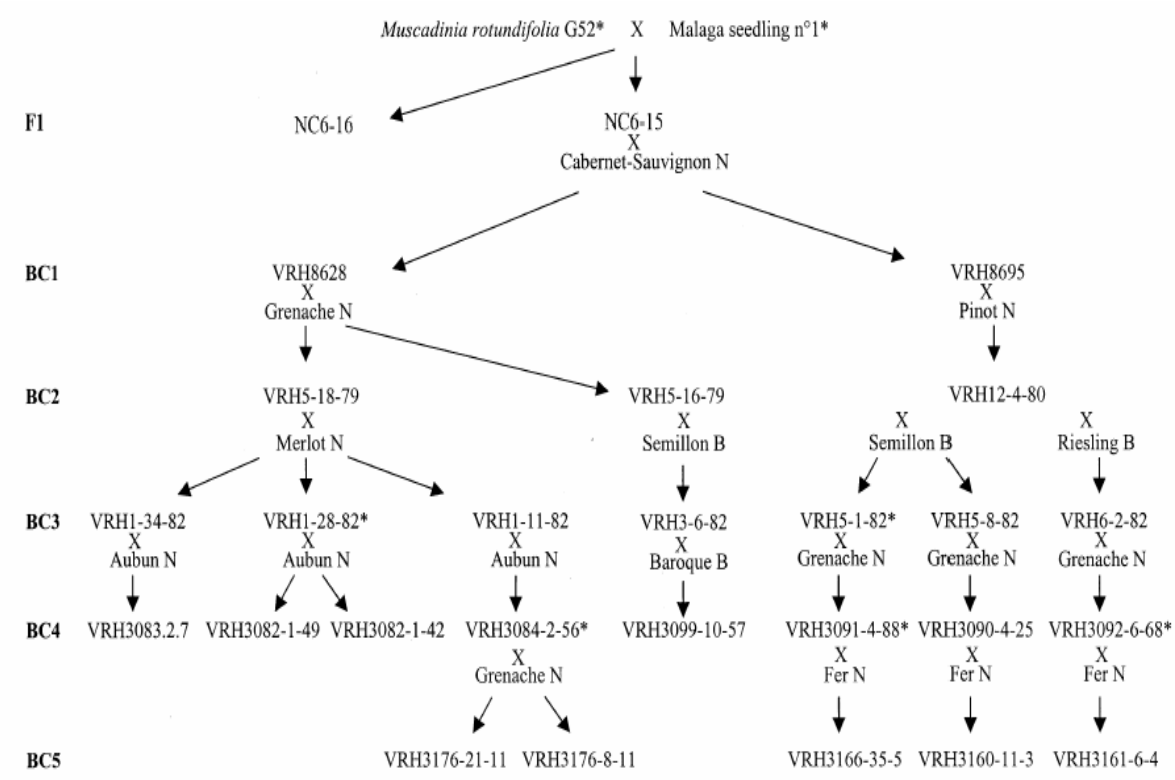

Figure 4. Production scheme of BC(n) families (PAUQUET *et al.* 2001).

Results and Conclusions

VMC1g3.2 proved to be unsuitable for genotyping the BC_3 selfed progeny. In the case of markers VMC8g9 and VVIm11 both RR and Rr genotypes appeared, but in unexpected ratios. New phenotyping and genotyping tests would be indispensable to explain these results.

Publications relevant to the topic

Results are not published yet.

MAPPING PM RESISTANCE GENE OF KISHMISH VATKANA WITH MOELCULAR MARKERS

Grapes of Eurasian origin were thought to be susceptible to American native pathogens like powdery and downy mildew. This assumption was explained by the geographical isolation of host and pathogen during their evolution. However, certain East Asian grape species, for example *Vitis piasezkii*, found to be at least partially resistant to PM (STAUDT 1997, KORBULY 1999). Genetic resources that contributed to *V. vinifera* cultivars are dispersed through Armenia, Iran, regions around the Black Sea and the countries of Central Asia (THIS et al 2006). These resources were studied and utilized only by regional breeders, and remained unknown for European and American grape breeders. FILIPENKO and STIN (1977) identified PM resistance in Dzhandzhal kara and used this accession in a breeding program in Russia. PM inoculation studies in the 1960's lead to the identification of nine resistant varieties out of 392 *V. vinifera* accessions originated from Armenia, Moldova, Russia, Georgia and Uzbekistan. One of these accessions, Kishmish vatkana, was reported by Kozma et al. (2006, 2009) free of PM infections under heavy disease pressure in the germplasm collection of the Uzbek Research Institute for Horticulture, Viticulture and Enology, Tashkent, Uzbekistan. In 1998 Kishmish vatkana was imported to Hungary. The variety, grown under field and greenhouse conditions in Hungary was confirmed to be resistant to the naturally occurring PM isolates in the absence of chemical disease control, only senescent leaves showed slight infection symptoms occasionally (Kozma et al. 2006).
In resistance breeding program lead by Pál Kozma for decades, Kishmish vatkana was crossed in the Research Institute for Viticulture and Enology with cultivars Nimrang and Kunbarát and the progeny (370 and 40 individuals, respectively) was phenotyped for powdery mildew symptoms in greenhouse. The segregation ratio proved to be 1:1 corresponding to a typical monohybrid test-cross suggesting the monogenic dominant nature of the resistance gene present in Kishmish vatkana in heterozygous status. The *REN*1 name was given to the resistance gene after the new terminology of the PM: **R**esistance to ***E**rysiphe **n**ecator* (Kozma et al. 2006). Mapping with molecular markers were based on these classical genetic results.

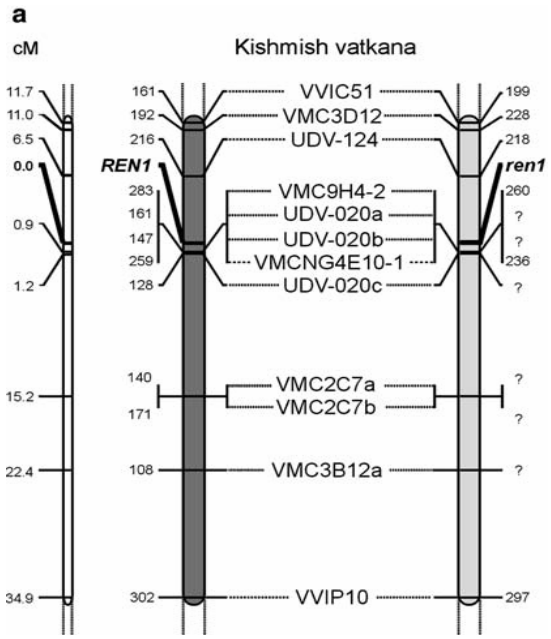

Figure 5: Position of REN1 and VMC9H4.2, VMCNG4E10.1 and UDV020 SSR markers in LG 13 in Kishmish vatkana (HOFFMANN et al. 2008).

ANALYSIS OF NIMRANG X KISHMIS VATKANA WITH *REN*1- LINKED SSR MARKERS

A progeny of 310 plants from a 'Nimrang' × 'Kishmish vatkana' cross were classified as susceptible or resistant by scoring for the presence or absence of visible conidiophores throughout two successive seasons. Phenotypic segregation revealed the presence of a single dominant allele (*REN*1) which was heterozygous in 'Kishmish vatkana'. A bulk segregant analysis was carried out using 15 individuals for each susceptible and resistant class and 195 SSR markers scattered across the entire genome. Association with the resistance trait was inferred by measuring the ratio of the peak intensities of the two alleles in the bulks for each marker heterozygous in 'Kishmish vatkana'. The phenotypic locus was assigned to linkage group 13, a genomic region in which no disease resistance had been reported previously. The *REN*1 position was restricted to a 7.4 cM interval by analyzing the 310 offspring for the segregation of markers that surrounded the target region. The closest markers, VMC9H4.2, VMCNG4E10.1 and UDV020, were located 0.9 cM from the *REN*1 locus (Figure 5).

Results and Conclusions

*REN*1 a new powdery mildew resistance gene was identified in Kishmish vatkana and localised to the LG13, proving its diversity from RUN1, the dominant PM gene of *Muscadinia rotundifolia*. VMC9H4.2, VMCNG4E10.1 and UDV020 SSR markers, being in allele association (linkage disequlibrium) with the resistance gene were determined around the locus applicable for MAS (marker assisted selection) purposes.

Publications relevant to the topic

Hoffmann S., Di Gaspero G., Kovács L., Howard S., Kiss E., Galbács Zs., Testolin R., Kozma P. 2008. Resistance to *Erysiphe necator* in the grapevine 'Kishmish vatkana' is controlled by a single locus through restriction of hyphal growth. Theoretical and Applied Genetics, 116:427-438.

ANALYSIS OF GÉNUAI ZAMATOS X KISHMIS VATKANA WITH *REN*1- LINKED SSR MARKERS

Based on the mapping results obtained with the Nimrang x Kishmish vatkana cross 78 PM symptomless and 68 infected samples the Génuai zamatos x Kishmis vatkana progeny were genotyped with the UDV20 marker. The marker genotypes of the susceptible and resistant plants are compiled in Table 3 showing that the resistant progeny of Génuai zamatos x Kishmish vatkana is supposed to possess a 164 bp REN1 linked marker alleles in UDV20 locus deriving from the Kishmish vatkana parent, since Génuai zamatos is a PM susceptible *V. vinifera* cultivar.

In 75 of 78 symptomless plants the 164 bp allele was detected, and only one of the susceptible plants carried this allele. The repetition of the artificial infection could confirm the reason for this discrepancy.

T a b l e 3

Results of Génuai zamatos x Kishmish vatkana progeny with the REN1 linked UDV20 marker (shaded number indicates the "resistant marker allele of Kishmish vatkana)

Variety	*REN1*
	UDV20
Génuai zamatos	138-148
Kishmish vatkana	138-164
Susceptible progeny	138-138 138-148
Symptomless progeny	138-164 148-164

Results and Conclusions

The UDV 20 marker is reliable in MAS, since the phenotype and genotype of the progeny yielded a 97% agreement taking into account that we cannot exclude the occurrence of phenotyping error.

Publications relevant to the topic

Katula-Debreceni D., Veres A., Szőke A., Lencsés A.K., Kozma P., Hoffmann S., Kiss E. 2010. Marker-based selection for powdery mildew resistance genes in different grape hybrid families. *Submitted.*

GENE PYRAMIDING: *RUN*1/ RPV1/*REN*1 ANALYSIS IN BC_4 x KISHMISH VATKANA PROGENY

Molecular marker-assisted selection (MAS) was performed to follow the inheritance of two separate powdery mildew-resistance loci, *RUN*1 and *REN*1, and a downy mildew resistance locus, *RPV1,* in the BC_4 x Kishmish vatkana hybrid grape population. The *REN*1 locus was introgressed from the powdery mildew-resistant *Vitis vinifera* L. variety Kishmish vatkana, whereas the *RUN*1 and *RPV*1 loci were introgressed from a *Muscadinia rotundifolia* x *V. vinifera* BC_4 hybrid plant derived from a recurrent pseudo-backcross breeding scheme (Figure 1). Using an F_1 hybrid progeny consisting of 411 plants and applying several SSR markers, we demonstrated that the powdery mildew-resistance phenotype co-segregated with the presence of at least one resistance locus-linked marker in the genome. Our data also corroborated earlier findings that the *M. rotundifolia*-derived *RUN*1 and *RPV*1*1* loci are closely linked. To further streamline the selection process, we developed a multiplex PCR- and agarose gel electrophoresis-based method for the simultaneous detection of both *RUN*1 and *REN*1. The results illustrate that MAS offers a rapid and accurate method to select hybrid genomes that combine multiple loci of interest in grape.

In the PTE Research Institute for Viticulture and Enology the (VRH 3082-1-42) BC_4 x Kishmish vatkana cross resulted in 1,185 progeny plants. Following natural infection under greenhouse conditions, 286 of these plants were determined to be PM-susceptible and 899 PM-resistant. For further analysis, first we randomly selected 411 plants from the 899 PM-resistant progeny, and 30 plants from the PM-susceptible progeny. We used these plants to test how PM-resistance co-segregated with *REN*1- and/or *RUN*1–specific markers. To find markers that can be used for routine genotyping in MAS, we evaluated several SSR markers

for each *REN*1- and *RUN*1. For *REN*1-linked markers, we applied VMC9h4.9, VMCNg4e10.1, and UDV020a, determined by mapping the *REN*1 gene (HOFFMANN et al. 2008) which are located at a genetic distance of approximately 0.9 cM from the *REN*1 locus. For all three of these markers, amplicon size differences allowed unambiguous distinction of *REN*1 and its homologous allele. Allele sizes for VMC9h4.2, VMCNg4e10.1, and UDV020a for the progeny are shown in Table 4. The three *REN*1-linked alleles were always inherited together, confirming their tight linkage as reported previously (HOFFMANN et al. 2008). All plants that inherited the *REN*1-linked markers were resistant to PM, and none of the 30 PM-susceptible plants inherited any of these markers. Of these, 154 plants harboured only *REN*1, and 146 contained both *REN*1 and *RUN*1*1* loci.

For *RUN*1-linked markers, we tested VMC8g9. We found that 111 of the 411 PM-resistant plants and none of the 30 PM-susceptible plants harboured a 160 bp allele at this locus. The allele sizes of VMC8g9 were 160 (*RUN*1-linked), 167, and 174 bp, and were readily distinguishable from one-another. Since we only had one informative *RUN*1-linked marker, we thought it important to confirm the VMC8g9-generated results with an independent method. Thus, we tested if this SSR marker co-segregated with *RUN*1-specific dominant markers CB69.70 and CB137.138, which had been designed on the basis of the BAC library clones by Barker and co-workers (2005). All plants that harboured the *RUN*1-linked VMC8g9 allele were also tested and were positive with CB69.70 and CB137.138. None of the 30 PM-susceptible progeny tested were positive for any of these four *RUN*1-linked alleles (KATULÁNÉ et al 2009).

Merdinoglu and co-workers (2003) showed that the *RPV*1 locus, conferring resistance to downy mildew, was tightly linked to the *RUN*1 locus in *M. rotundifolia* x *V. vinifera* BC_2 hybrid plants. They were able to link the *RPV*1 locus to SSR marker VMC1g3.2. The *RPV*1-specific allele size of VMC1g3.2 is 122 bp. As the VMC1g3.2 primers also prime the synthesis of a 122-bp amplicon in Kishmish vatkana, this marker can not be used to follow the segregation *RPV*1 locus in heterozygous individuals in this population. Nonetheless, we determined that individuals that are homozygous for this allele (122:122, *RPV*1*+)* are also *RUN*1+, which corroborates the findings by Merdinoglu and co-workers (2003). To follow the segregation of the *RPV*1*1* locus, we used VVIm11 as a new primer. However the entire segregating population will also need to be evaluated for downy mildew resistance and susceptibility.
SSR results of the resistance gene linked markers are presented in Table 4. In the BC_4 x Kishmish vatkana hybrid family the BC_4 parent is heterozygous for the *M. rotundifolia RUN1*/*RPV1* genes, therefore the alleles 160 bp, 294 bp or 122-122 genotype (with markers VMC8g9, VVim11, VMC1g3.2, respectively) indicate the presence of the resistance genes. In Kishmish vatkana, which is heterozygous for the *REN1* locus, genotypes of 260-260 and alleles 286 bp, 164 bp are the markers for PM resistance (with markers VMCNg4e10.1, VMC9h4.2 and UDV20, respectively). Among 441 symptomless individuals all the expected genotypes: *RUN1*/*RPV1*/*REN1, RUN*/*RPV1*, *REN1* could be identified. Figure 6 shows the results obtained by DNA fragment analysis (ALF-pattern: separation of PCR product by ALFExpress analyzator).

T a b l e 4

SSR Marker results in BC4 x Kishmish vatkana hybrid family (shaded number indicates the "resistant marker allele" of *Muscadinia rotundifolia* and *V.vinifera* cv. Kishmish vatkana)

Variety	*RUN1/RPV1*			*REN1*		
	VMC8g9	VVIm11	VMC1g3.2	VMCNg4e10.1	VMC9h4.2	UDV20
BC_4	160-167	272-294	122-142	260-260	282-298	148-148
Kishmish vatkana	167-174	260-284	122-142	240-260	262-286	138-164
Susceptible progeny	167-167 167-174	272-284 260-272	122-142 142-142	240-260	262-282 262-298	138-148
Symptomless progeny	160-167 160-174	260-294 284-294	122-142 122-122	260-260	282-286 286-298	148-164

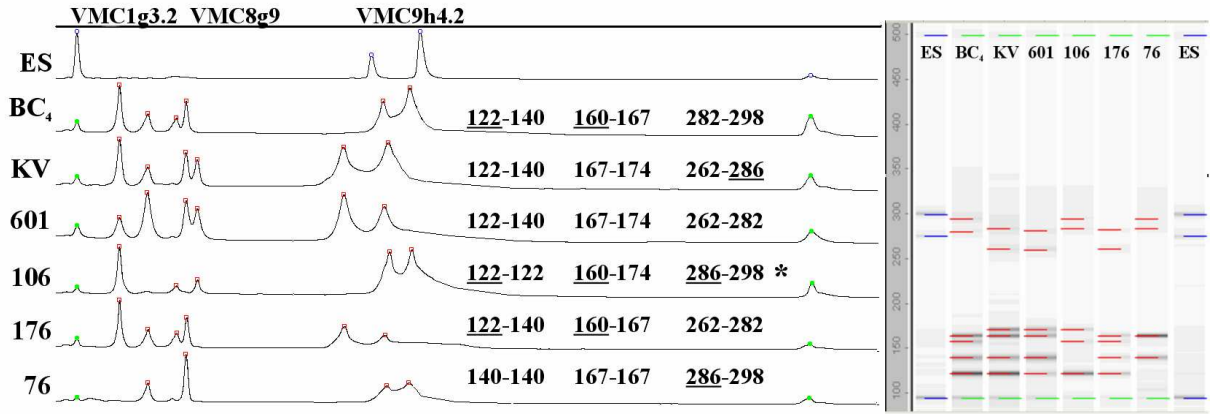

Figure 6 ALF-pattern of multiplex PCR using VMC1g3.2, VMC8g9 and VMC9h4.2 primers.

Left part: ES: external standard (95 bp, 275 bp, 300 bp, 500 bp), BC_4 (*Run1+/Ren1-*), KV: Kishmish vatkana (*Run1-/Ren1+)*, BC_5 progeny: 601: *Run1-/Ren1-,* 106: *Run1+/Ren1+,* 176: *Run1+/Ren1-,* 76: *Run1-/Ren1+*. The *Run1+/Ren1+* genotype is labelled with *. Right part: Virtual gel photo of the multiplex PCR.

Having verified markers that reliably co-segregated with the *REN*1 and *RUN*1 loci, we set out to identify a marker combination that would enable us to simultaneously detect the presence of both resistance loci in a multiplex PCR reaction. Such markers need to generate PCR products that differ in size, but anneal to genomic DNA at similar temperature. We determined that the following marker combinations were suitable for multiplex PCR: VMC9h4.2/VMC8g9, VMCNg4e10.1/VMC8g9, VMC1g3.2/VMC9h4.2, and VMC1g3.2/VMCNg4e10.1 and VMC1g3.2/VMC9h4.2/VMC8g9 (Figure 6). We previously reported that homologues of the VMC8g9 marker could be distinguished in a different *M. rotundifolia* x *V. vinifera* BC_5 hybrid progeny when electrophoresed in high-concentration

agarose gels (Molnár et al. 2007). Thus, we generated multiplex PCR products with VMC8g9 and VMC9h4.2 primers in individuals Pecs-06-1/601, Pecs-06-1/090, Pecs-06-1/006, and Pecs-06-1/036, which represented *RUN*1-/*REN*1-, *RUN*1+/*REN*1+, *RUN*1+/*REN*1-, and *RUN*1-/*REN*1+ genotype*s*, respectively. We then fractionated the PCR products in 1.2% agarose gel and 4% high-resolution MetaPhor® agarose gels. While the 1.2% routine agarose is suitable for separating the resistant genotypes from the sensitive ones only with VMC1g3.2, we could reliably detect the various allele sizes with VMC8g9 and VMC9h4.2 in 4% MetaPhor® agarose gel (Figure 7a). Figure 7b demonstrates these results in a barcode format.

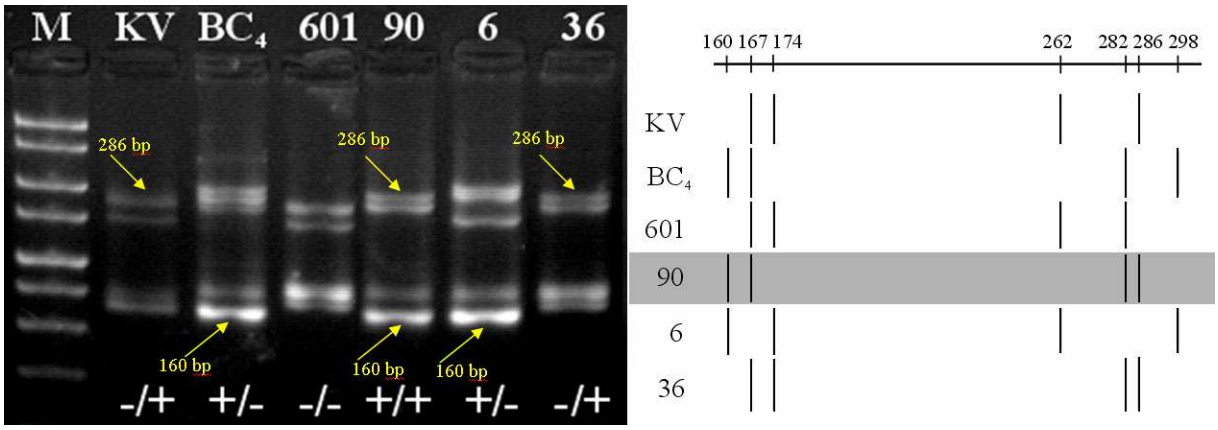

Figure 7. Left part: Multiplex PCR products (using VMC8g9 and VMC9h4.2 primers) separated in 4% MetaPhor® agarose gel. M: DNA molecular weight marker (BioLine HyperLadder V., Izinta Kft, Budapest, Hungary); KV: Kishmish vatkana: *Run1-/Ren1+*; BC_4: *Run1+/ Ren1-;* BC_5 progeny: 601: *Run1-/Ren1-* 90: *Run1+/Ren1+,* 6: *Run1+/Ren1-,* 36: *Run1-/Ren1+*. Right part: **Barcode of multiplex PCR results.** The genotype containing both powdery mildew resistance genes is shaded.

In summary we analyzed all the 899 symptomless individuals of the BC_4 x Kishmish vatkana progeny and identified the expected *RUN*1-/*REN*1-, *RUN*1+/*REN*1+, *RUN*1+/*REN*1-, and *RUN*1-/*REN*1+, where *RPV*1± genotypes were taken identical to *RUN*1±, however we found possible recombinants between VMC8g9 and VMC1g3.2 (data not shown). Further molecular analyses genotyping supprted by the grape physical map (Jaillon et al. 2007), repetition of PM infection, precise phenotyping of both PM and DM symptoms are indispensable to find recombinants in the progeny.

Results and Conclusions

We demonstrated that SSR markers developed previously for the mapping of these disease resistance loci in grape can be reproducible for MAS. Moreover, we showed that the *REN*1- and *RUN*1-linked markers consistently co-segregated with the PM resistance, indicating that they can be reliably used in MAS. With these markers we were able to prove the presence of the pyramided PM resistance genes in the BC_5 hybrid family. Plants carrying pyramided resistance genes *(RUN*1*, REN*1*)* for the same phenotype can be identified only with DNA level analysis. This is the first time when SSR markers linked to *REN*1 were used for MAS.

We elaborated a multiplex PCR method for MAS of pyramided resistance genes We determined that the following marker combinations were suitable for multiplex PCR: VMC9h4.2/VMC8g9, VMCNg4e10.1/VMC8g9, VMC1g3.2/VMC9h4.2, VMC1g3.2/VMCNg4e10.1 and VMC1g3.2/VMC9h4.2/VMC8g9. Setting up an agarose gel electrophoresis system replacing for the ALFExpress instrumental analyses in the selection

process. As results of the MAS 200 plants of *RUN*1/*RPV*1/*REN*1 genotype were planted into the field in PTE Research Institute of Viticulture and Enology, Pécs.

Publications relevant to the topic

Katuláné Debreceni D., Lencsés A. K., Szőke A., Veres A., Hoffmann S., Erdélyi Sz., Heszky L., Kiss E., Kozma P. 2009. *Muscadinia rotundifolia* (Mich.) Small és vitis vinifera eredetű lisztharmat rezisztencia felhasználása szőlő nemesítésben markerekre alapozott szelekcióval. Kertgazdaság 41 (2): 82-91.

Katula-Debreceni D., Lencsés A. K., Szőke A., Veres A., Hoffmann S., Kozma P., Kovács L.G., Heszky L., Kiss E. 2010. Marker-based selection for powdery mildew resistance genes in different grape hybrid families. Submitted.

GENOTYPING OF 120 GRAPEVINE VARIETIES IN 17 SSR LOCI

Results of genotyping of 115 grapevine cultivars including Carpathian basin (HALÁSZ et al. 2005), regional European, international, Central-Asian, "new" Hungarian cultivars in 12 SSR loci were published by Galbács et al. (2009). The 12 loci are as follows: Scu08, Scu10, VrZag47, **VrZag62**, **VrZag79**, VrZag83, VrZag112, VVMD21, **VVMD25, VVMD28**, VVMD31, VVMD36. Additional 5 markers **VVMD5, VVMD7, VVMD27, VVMD32** and **VVS2** recommended by GrapeGen06 project were valso applied as secondary descriptors of grape cultivars. Data obtained in 9 loci (**bold**) are uploaded in the European Vitis Database. Based on the SSR fingerprints the cultivars can be discriminated except berry colour variants (concultas) of several varieties.

Results and conclusions

The SSR allele size data were converted into barcodes, enabling a simple visual comparison of the DNA fingerprints. Neither the first 12 nor the additional 5 markers generated special, exclusive alleles in PM resistant cultivars Kishmish vatkana and Dzhandzhal kara.

Publication relevant to the topic

Galbács Zs., Molnár S., Halász G., Hoffmann S., Galli Zs., Szőke A., Veres A., Heszky L., Kozma P., Kiss E. 2007. „DNS-ampelográfia": Szőlőfajták jellemzése mikroszatellit vonalkóddal. Agrár- és Vidékfejlesztési Szemle, 2(2): 93-99.

Galbács Zs., Molnár S., Halász G., Hoffmann S., Kozma P. Kovács L., Veres A., Galli Zs., Szőke A., Heszky L. Kiss E. 2009. Identification of grapevine cultivars using microsatellite-based DNA barcodes. Vitis 48(1): 17-24.

GENOTYPING OF GRAPEVINE CULTIVARS WITH *RUN*1 AND *REN*1 LINKED MARKERS

We determined the SSR profile of several selected cultivars (old and new) with markers linked to PM and PM loci in resistant varieties BC4, Kishmish vatkana and Dzhandzhal kara.

As it can be seen from the table none of the 13 cultivars carries the alleles linked either to *RUN*1/*RPV*1 or *REN*1. At the same time Kishmish vatkana and Dzhandzhal kara share common alleles in loci VMC9h4.2 and UDV20 loci, suggesting together with the results of the PD 72424 project that these two cultivars possess the same *REN*1 PM resistance gene confirmed by other COLEMAN et al. (2010).

T a b l e 5

Results of PM6DM resistant varieties Kishmis vatkana, Dzhandzhal kara, BC_4 and several sensitive cultivars with the *RUN*1/*RPV*1 and *REN*1 linked SSR markers (shaded numbers show the allele data present only the resistant varieties.

	*RUN*1/RPV1			*REN*1	
Variety	VMC8g9	VMC1g3.2	VVIm11	VMC9h4.2	UDV20
BC_4	**160**:167	**122**:142	270:**298**	282:298	148:148
Kishmis vatkana	167:174	122:142	260:284	262:**286**	(128):138:(148):**164**
Dzandzhal kara	167:174	122:128	-	282: **286**	(128):138:(148):**164**
Cardinal	179:179	135:142	278:284	289:307	138:148:152:158
Csabagyöngye	179:179	118:135	284:284	264:289	138:152:162
Irsai Olivér	179:202	118:140	278:284	289:312	138:152
Madeleine angevine	176.179	118:128	284:292	289:289	138:152
Muscat Fleur d'Oranger	179:205	128:135	280:284	264:312	138:162
Kadarka	179:179	140:140	278:278	289:307	135:148:158
Pozsonyi	167:202	128:140	278:278	282:312	138:148:162
Kossuth szőlő	176:179	118:128	284:292	289:289	138:152
Duchess of Buccleugh	164:205	128:128	280:292	264:282	138:148:162
Izsáki	167:174	118:128	280:284	262:262	128:152:162
Kövérszőlő	174:179	128:135	280:284	276:276	138:160
Leányka	167:172	128:128	280:292	282:282	128:138:152
Királyleányka	172:176	128:128	270:280	289:289	128:135:152:158

Results and conclusions

BC_4 can be characterized with a 160 and 298 bp allele in VMC8g9 and VVIm11 loci (*RUN*1/*RPV*1 markers, while in the case of Kismish vatkana and Dzhandzhal kara a 286 and a 164 bp fragment amplified with VMC9h4.2 and UDV20 primers, respectively missing from the other 13 non PM resistant varieties. SSR profiles in REN1 linked loci suggest that Kismish vatkana and Dzhandzhal kara possess the same *REN*1 PM resistance gene.

Publication relevant to the topic

Results are not published yet.

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